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Biocatalytic dearomatisation of *para*-fluorobenzoic acid

Access to versatile homochiral building blocks with quaternary centres

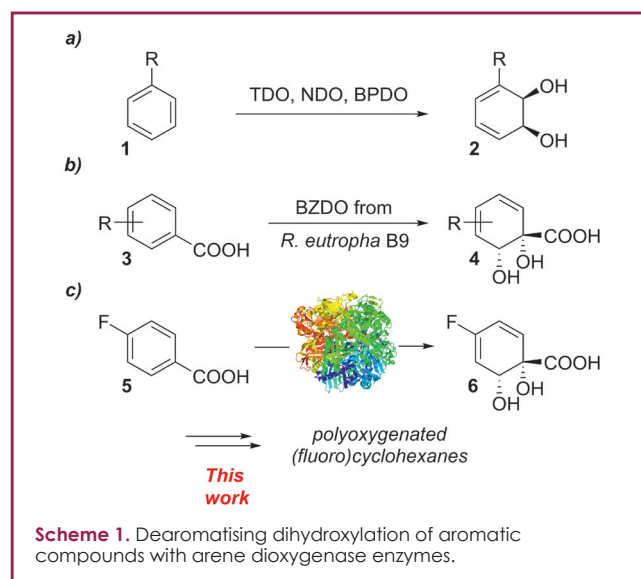
KEYWORDS: Benzoate dioxygenase, dearomatisation, arene cis-diols, fluoroalkenes, green chemistry.

Abstract The enzyme benzoate dioxygenase (BZDO) from *Ralstonia eutropha* B9 is able to dihydroxylate benzoic acids in a dearomative process that proceeds with a different regioselectivity than other known dioxygenase enzymes. Here we show that 4-fluorobenzoic acid is oxidised by BZDO to give an enantiopure diol that can be rapidly elaborated to highly oxygenated homochiral building blocks with quaternary centres. Notably, the diol produced in this biotransformation displays reactivity which is distinct from that of the more extensively studied non-fluorinated analogue.

INTRODUCTION

The dearomatising dihydroxylation of an aromatic ring is a reaction which is very difficult to perform by conventional chemistry (1). However, arene dioxygenase enzymes are able to effect this chemistry with great stereo- and regioselectivity (2-8). Arene dioxygenases are produced by various microorganisms and the most extensively studied enzymes to date have been toluene dioxygenase (TDO), naphthalene dioxygenase (NDO) and biphenyl dioxygenase (BPDO). All of these possess a wide substrate scope, and their selectivity towards substituted arenes has been studied (9). Thus, a monosubstituted arene **1** will typically be oxidised to give a cyclohexadiene *cis*-diol of type **2**, wherein hydroxylation has occurred *ortho* and *meta* to the original substituent, and with the absolute stereochemistry as shown (Scheme 1a). In contrast, benzoate dioxygenase (BZDO) exhibits a distinct selectivity and is able to effect the oxidation of benzoic acids **3** to give products of type **4**. In *cis*-diols **4**, the hydroxylation occurs *ipso* and *ortho* to the carboxylic acid substituent (Scheme 1b), and with the absolute stereochemistry as shown (i.e. the opposite sense of stereoinduction compared to the formation of **2**). The densely packed, differentiated functionality in diols of type **4** makes them ideal building blocks for complex molecule synthesis. The parent diol (**4**, R = H), derived from unsubstituted benzoic acid (**10**) has been used in various syntheses, for example of drug candidates (11-14), natural products (15-20) and carbohydrates (21-24). In contrast, substituted diols (**4**, R ≠ H) have scarcely been exploited to date, despite the substrate scope of the enzyme having been determined (25). For wild-type BZDO, substituents *meta* to the carboxylic acid group in **3** are best tolerated; we have previously reported production of **4** (R = 3-bromo) and demonstrated the use of the pendant bromine atom as a

handle for further functionalisation (26). Also, production of **4** (R = 3-ethyl) has been reported (27). To the best of our knowledge, no *para* substituted diols of type **4** have been used in synthesis to date, although the kinetic parameters for BZDO-mediated oxidation of *para*-fluorobenzoic acid (**5**) to diol **6** have been determined (28). In this paper we describe the production of **6** and its elaboration to various highly oxygenated structures (Scheme 1c). An early report on the synthetic utility of **4** (R = H) showed that each position on the ring was functionalisable selectively through short reaction sequences (29). We have found that many of these reactions give a different outcome when applied to fluorinated metabolite **6**.



MATERIALS AND METHODS

Biotransformation of 4-fluorobenzoic acid (**5**) to (1*S*,6*R*)-4-fluoro-1,6-dihydroxycyclohexa-2,4-diene-1-carboxylic acid (**6**)

A sterile pipette tip was streaked across a frozen (-80 °C) glycerol stock solution of *Ralstonia eutropha* B9 and added to a sterile solution of 100 mL of Hutner's mineral base (30) and D-fructose (2.0 mL, 10% w/v). The solution was placed on an orbital shaker (250 rpm) at 27 °C and shaken for 72 h. The resulting slightly cloudy culture was added to Hutner's mineral base (1.5 L), which was maintained at ca. 30 °C and through which air was continuously sparged. D-fructose solution (10 mL, 10% w/v) was then added. A further quantity (30 mL) was added over 24 h via syringe pump. The culture was then induced with sodium 4-fluorobenzoate solution (1.5 M, 5.0 mL) and a further quantity of D-fructose solution was added over 24 h (40 mL). Product formation was then assayed by ¹⁹F-NMR analysis of the culture. Thus, an aliquot of the culture was removed, filtered through a Whatman PuraDisc FP-30 0.45 µm syringe filter, then a ¹⁹F NMR spectrum of the filtrate was acquired directly. Chemical shifts for the starting material **5** and product **6** were observed to drift slightly between each assay, with the resonance for **5** being observed between δ -117.84 and -116.67 ppm, whereas the resonance for **6** was observed between δ -122.81 and -123.98 ppm (the signal for **6** was always upfield of the signal for **5** by 6.14 ppm). NMR showed induction to have been successful (absence of starting material **5**). Substrate **5** and carbon source (D-fructose) were introduced continuously over 5 days, with periodic assaying of conversion by ¹⁹F-NMR. In total, 29 mL of sodium 4-fluorobenzoate solution and 145 mL D-fructose solution were added to the culture. The culture was then centrifuged (5000 rpm, 45 minutes) and the supernatant collected. The solvent was then removed under vacuum at 30 °C to give a green solid. This solid was found by ¹H-NMR to contain the desired product, salts and D-fructose. Thus, the material was redissolved in water (80 mL), to which isopropanol (800 mL) was added. After 10 min, the cloudy solution was decanted off, leaving behind a brown sludge. A further quantity of water (80 mL) was added to the brown sludge, and isopropanol (800 mL) added. Again, the cloudy solution was decanted off leaving a brown sludge behind. The decanted solutions were cooled to 4 °C for 1 h to aid precipitation of the solid. The solid was then filtered off and dried under vacuum to give a cream coloured solid, which contained **6** as the mixed sodium/potassium carboxylate salt and a variable proportion of growth medium salts. 1.35 g of solid was isolated, whereas the concentration of the starting material in the culture was ≈ 3.2 g/L.

Methyl (3*aS*,7*aR*)-6-fluoro-2,2-dimethylbenzo[d][1,3]dioxole-3*a*(7*aH*)-carboxylate (**7**)

To a suspension of impure *cis*-diol carboxylate mixed sodium/potassium salt **6-Na/K** (0.287 g, also contains growth salts) in 2,2-dimethoxypropane (10 mL), cooled to ~0 °C, was added trifluoroacetic acid (TFA) (0.56 mL, 7.32 mmol) dropwise via syringe pump. Following addition of TFA, the reaction was allowed to warm to room temperature overnight. The suspension was then filtered through a pad of Celite™ and the solution concentrated under vacuum, before being dispersed in H₂O (20 mL) and extracted with CH₂Cl₂ (2 × 20 mL). The organic layers were combined, dried over MgSO₄ and the solvent removed to give a dark brown oil. This brown oil was re-dissolved in CH₂Cl₂ and the solvent again

removed under vacuum to aid excess TFA removal.

This was repeated several times. The crude product was re-dissolved in a mixture of MeOH (7.5 mL) and benzene (7.5 mL). Trimethylsilyldiazomethane was added slowly to the resulting solution until a yellow colour persisted and effervescence ceased. The solvent was removed to give a brown oil, which was purified by column chromatography (5% EtOAc, 95% petroleum ether) to give a pale yellow liquid **7** (0.216 g, 0.947 mmol). *R*_f = 0.3 (10% EtOAc, 90% petroleum ether); [α]_D²⁴ = -242 (c 1.00, CH₂Cl₂); ¹H NMR (500 MHz, CDCl₃) δ = 6.01 (1H, ddd, *J* = 10.0, 7.5, 2.0 Hz), 5.91 (1H, ddt, *J* = 10.0, 5.0, 1.0 Hz), 5.48 (1H, dddd, *J* = 11.0, 5.0, 2.0, 1.0 Hz), 5.05 (1H, ddd, *J* = 6.0, 5.0, 1.0 Hz), 3.78 (3H, s), 1.43 (3H, s), 1.41 (3H, s); ¹³C NMR (126 MHz, CDCl₃) δ = 171.2 (s), 157.8 (d, *J* = 254.5 Hz), 129.8 (d, *J* = 10.0 Hz), 121.1 (d, *J* = 38.5 Hz), 107.5 (s), 99.1 (d, *J* = 18.0 Hz), 79.6 (d, *J* = 2.0 Hz), 74.0 (d, *J* = 12.5 Hz), 53.2 (s), 27.0 (s), 25.0 (s); ¹⁹F NMR (500 MHz, CDCl₃) δ = -111.7 (dq, *J* = 11.5, 6.0 Hz); *v*_{max} (neat) 2991, 2957, 1737, 1683, 1252, 1040, 802 cm⁻¹; HRMS (ESI+) *m/z* calculated for (C₁₁H₁₃O₄FN₂)⁺ 251.0690, found 251.0707.

Methyl (3*aS*,4*R*,5*S*,7*aR*)-6-fluoro-4,5-dihydroxy-2,2-dimethyl-5,7*a*-dihydrobenzo[d][1,3]dioxole-3*a*(4*H*)-carboxylate (**8**) and methyl (5*S*)-7-hydroxy-2,2-dimethyl-6-oxobenzo[d][1,3]dioxole-3*a*(6*H*)-carboxylate (**9**)

To a solution of diene **7** (46 mg, 0.202 mmol, 1 eq.) in acetone was added *N*-methylmorpholine-*N*-oxide (NMO) (26 mg, 0.222 mmol, 1.1 eq.). H₂O (ca. 0.2 mL) was added until full dissolution of the NMO was observed, and OsO₄ (2.5%wt in *t*-BuOH, 0.10 mL, 10.1 µmol, 5 mol%) added. The solution was stirred overnight and turned dark brown in colour. The reaction was quenched with addition of aq. Na₂S₂O₃ (1M, 10 mL) followed by aq. sat. NaHCO₃ (5 mL). The product was extracted with EtOAc (3 × 15 mL). Organic layers were combined, washed with brine (30 mL), dried over MgSO₄ and the solvent removed to give a dark brown oil, which was a mixture of 2 different products. These were separated by column chromatography (30% EtOAc, 70% petroleum ether to 50% EtOAc, 50% petroleum ether) to give a white solid **8** (27 mg, 0.103 mmol, 51%) and a yellow oil **9** (4 mg, 16.7 µmol, 6%). Data for **8**: *R*_f = 0.3 (50% EtOAc, 50% petroleum ether); [α]_D²⁴ = -24 (c 0.25, CH₂Cl₂); ¹H NMR (500 MHz, CDCl₃) δ = 5.52 (1H, dd, *J* = 14.0, 4.5 Hz), 5.01 (1H, t, *J* = 5.0 Hz), 4.42-4.36 (1H, m), 4.29-4.24 (1H, m), 3.86 (3H, s), 3.63 (1H, d, *J* = 9.0 Hz), 3.10-3.02 (1H, m), 1.50 (3H, s), 1.41 (3H, s); ¹³C NMR (126 MHz, CDCl₃) δ = 172.9 (s), 159.6 (d, *J* = 269.0 Hz), 111.8 (s), 102.2 (d, *J* = 17.5 Hz), 83.8 (d, *J* = 2.0 Hz), 74.0 (d, *J* = 13.0 Hz), 72.4 (d, *J* = 8.0 Hz), 65.3 (d, *J* = 24.5 Hz), 53.4 (s), 27.9 (s), 25.8 (s); ¹⁹F NMR (500 MHz, CDCl₃) δ = -110.1 -- -110.4 (m); *v*_{max} (neat) 3405, 3327, 2991, 2950, 1748, 1703, 1257, 1241, 1088, 1053, 857 cm⁻¹; HRMS (ESI+) *m/z* calculated for (C₁₁H₁₅O₆FN₂)⁺ 285.0745, found 285.0728. Data for **9**: *R*_f = 0.2 (30% EtOAc, 70% petroleum ether); [α]_D²⁶ = -120 (c 0.20, CH₂Cl₂); ¹H NMR (500 MHz, CDCl₃) δ = 6.88 (1H, d, *J* = 9.7 Hz), 6.34 (1H, d, *J* = 9.7 Hz), 5.67 (1H, s), 3.77 (3H, s), 1.66 (3H, s), 1.61 (3H, s); ¹³C NMR (126 MHz, CDCl₃) δ = 183.0, 169.0, 145.8, 137.8, 129.9, 128.6, 120.1, 83.4, 54.2, 28.6, 25.7; *v*_{max} (film) 3392, 3000, 2956, 1744, 1649, 1598, 1380, 1220, 1140, 1111, 823 cm⁻¹; HRMS (ESI+) *m/z* calculated for (C₁₁H₁₂O₆Na)⁺ 263.0526, found 263.0537.

Methyl (3*aS*,5*aR*,8*aR*,8*bR*)-4-fluoro-2,2,7,7-tetramethyl-3*a*,8*b*-dihydrobenzo[1,2-*d*:3,4-*d'*]bis([1,3]dioxole)-8*a*(5*aH*)-carboxylate **15**

Diol **8** (31 mg, 0.122 mmol, 1.0 eq.) was dissolved in 2,2-dimethoxypropane (5.0 mL) and *p*-toluenesulfonic acid (pTSA) added (monohydrate, 3 mg, 15.8 µmol, 0.13 eq.). The

solution was stirred for 48 h. Aqueous saturated NaHCO_3 (15 mL) was added and the product extracted with CH_2Cl_2 (3 x 15 mL). The organic phases were combined, dried over MgSO_4 and the solvent removed. The resulting oil was purified by column chromatography (20% EtOAc, 80% petroleum ether) to give 15 as a colourless oil (30 mg, 99.3 μmol , 81%). R_f = 0.3 (20% EtOAc, 80% petroleum ether); $[\alpha]_D^{24}$ = -34 (c 1.00, CH_2Cl_2); ^1H NMR (500 MHz, CDCl_3) δ = 5.43 (1H, ddd, J = 6.2, 3.3, 1.5 Hz), 5.31 (1H, dd, J = 15.1, 3.3 Hz), 4.76 (1H, t, J = 5.9 Hz), 4.70 (1H, dd, J = 5.6, 1.5 Hz), 3.85 (3H, s), 1.39 (3H, s), 1.36 (3H, s), 1.35 (3H, s), 1.30 (3H, s); ^{13}C NMR (126 MHz, CDCl_3) δ = 170.9 (s), 156.2 (d, J = 265.1 Hz), 111.55 (s), 111.53 (s), 103.9 (d, J = 14.0 Hz), 82.3 (d, J = 2.1 Hz), 75.8 (d, J = 7.2 Hz), 73.1 (d, J = 11.0 Hz), 70.1 (d, J = 24.0), 53.2 (s), 28.3 (s), 27.84 (s), 27.78 (s), 26.5 (s); ^{19}F NMR (500 MHz, CDCl_3) δ = -117.7 (dt, J = 15.1, 6.2 Hz); ν_{max} (film) 2991, 2943, 1747, 1708, 1372, 1223, 1069, 1050, 851 cm^{-1} ; HRMS (ESI+) m/z calculated for $(\text{C}_{14}\text{H}_{19}\text{O}_6\text{FNa})^+$ 325.1058, found 325.1076.

((3aS,5aR,8aR,8bR)-4-Fluoro-2,2,7,7-tetramethyl-3a,8b-dihydrobenzo[1,2-d:3,4-d']bis([1,3]dioxole)-8a(5aH)-yl)methanol 16

Methyl ester 15 (18 mg, 59.6 μmol , 1 eq.) was dissolved in anhydrous THF (5.0 mL) and the solution was cooled to 0 $^{\circ}\text{C}$. LiBH_4 (4.0 M, 0.03 mL, 0.119 mmol, 2 eq.) was added dropwise and the solution allowed to warm to room temperature overnight. EtOAc (15 mL) was added slowly, followed by water (15 mL). The organic layer was collected and the product further extracted from the aqueous layer with EtOAc (2 x 15 mL). Organic layers were combined, washed with brine (20 mL), dried over MgSO_4 and the solvent removed. The resulting oil was purified by column chromatography (30% EtOAc, 70% petroleum ether) to give 16 as a colourless oil (15 mg, 54.7 μmol , 92%). R_f = 0.3 (30% EtOAc, 70% petroleum ether); $[\alpha]_D^{24}$ = -26 (c 5.0, CH_2Cl_2); ^1H NMR (500 MHz, CDCl_3) δ = 5.33 (1H, dd, J = 15.2, 3.3 Hz), 4.70 (1H, dd, J = 6.6, 5.2 Hz), 4.68-4.64 (2H, m), 3.86 (1H, t, J = 10.6 Hz), 3.79 (1H, d, J = 11.4 Hz), 2.25 (1H, d, J = 9.7 Hz), 1.45 (3H, s), 1.41 (6H, s), 1.36 (3H, s); ^{13}C NMR (126 MHz, CDCl_3) δ = 157.3 (d, J = 266 Hz), 110.7 (s), 111.0 (s), 103.8 (d, J = 14.2), 81.6 (s), 75.6 (d, J = 7.3 Hz), 73.6 (d, J = 10.9 Hz), 70.1 (d, J = 22.8), 64.6 (s), 29.8 (s), 28.8 (s), 27.9 (s), 26.6 (s); ^{19}F NMR (500 MHz, CDCl_3) δ = -117.0 (dt, J = 15.6, 6.3 Hz); ν_{max} (film) 3511, 2990, 2937, 1706, 1371, 1230, 1174, 1085, 1042, 917, 859 cm^{-1} ; HRMS (ESI+) m/z calculated for $(\text{C}_{13}\text{H}_{19}\text{O}_5\text{FNa})^+$ 297.1109, found 297.1122.

Methyl (3aS,5aR,6aR,6bR)-5a-fluoro-2,2-dimethyl-6a,6b-dihydroxireno[2',3':3,4]benzo[1,2-d][1,3]dioxole-3a(5aH)-carboxylate 17

Diene 7 (0.031 g, 0.136 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (3.00 mL) and the solution was cooled to -20 $^{\circ}\text{C}$. *meta*-chloroperbenzoic acid (*m*CPBA) (77% pure, 0.040 g, 0.177 mmol, 1.3 eq.) was added and the solution was allowed to warm to room temperature overnight. The reaction was quenched with the addition of aqueous $\text{Na}_2\text{S}_2\text{O}_3$ (1.0 M, 10 mL) and the product extracted with CH_2Cl_2 (3 x 10 mL). Organic layers were combined and washed with a saturated aqueous solution of NaHCO_3 (15 mL), before being dried over MgSO_4 . The solvent was removed to give a yellow/orange liquid, which was purified by column chromatography (10% EtOAc, 90% petroleum ether) to give 17 as a colourless liquid (26 mg, 0.107 mmol, 79%). R_f = 0.4 (20% EtOAc, 80% petroleum ether); $[\alpha]_D^{24}$ = -64 (c 0.25, CH_2Cl_2); ^1H NMR (500 MHz, CDCl_3) δ = 6.29 (1H, dd, J = 10.5, 4.0 Hz), 5.89 (1H, ddd, J = 10.5, 5.5, 1.0 Hz), 5.05 (1H, dt, J = 5.0, 1.5 Hz), 3.90 (1H, t, J = 1.5 Hz), 3.78 (3H, s), 1.46 (3H, s), 1.44 (3H, s); ^{13}C NMR (126 MHz, CDCl_3) δ = 170.0 (s), 131.6 (d, J = 13.0 Hz), 123.5 (d, J = 41.0 Hz), 112.9 (s), 89.9 (d, J = 262.0 Hz), 79.8 (s), 74.2 (d, J = 4.0

Hz), 53.6 (d, J = 17.5 Hz), 53.4 (s), 27.6 (s), 25.7 (s); ^{19}F NMR (500 MHz, CDCl_3) δ = -148.0 (q, J = 5.0 Hz); ν_{max} (film) 2992, 2957, 1745, 1378, 1263, 1192, 1057, 969 cm^{-1} ; HRMS (ESI+) m/z calculated for $(\text{C}_{11}\text{H}_{13}\text{O}_5\text{FNa})^+$ 267.0639, found 267.0649.

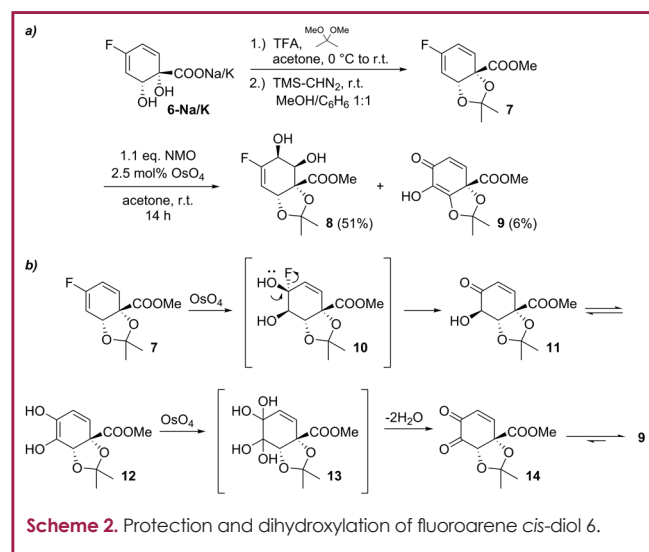
Methyl (3aS,4R,5S,5aR,6aR,6bR)-5a-fluoro-4,5-dihydroxy-2,2-dimethyltetrahydroxireno[2',3':3,4]benzo[1,2-d][1,3]dioxole-3a(4H)-carboxylate 18

Diol 8 (37 mg, 0.141 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (5.00 mL) and *m*CPBA (100% pure, 37 mg, 0.212 mmol, 1.5 eq.) added. [CAUTION: 100% *m*CPBA is a contact explosive]. After 24 h, a further quantity of *m*CPBA (100%, 37 mg, 0.212 mmol, 1.5 eq.) was added. The solution was stirred for 5 days. Aqueous $\text{Na}_2\text{S}_2\text{O}_3$ (1.0 M, 10 mL) was added, and the product extracted with CH_2Cl_2 (3 x 15 mL). Organic layers were combined, dried over MgSO_4 and the solvent removed. The resulting solid was purified by column chromatography (50% EtOAc, 50% petroleum ether) to give 18 as a white solid (22 mg, 79.1 μmol , 56%). R_f = 0.3 (50% EtOAc, 50% petroleum ether); $[\alpha]_D^{24}$ = +9.33 (c 0.75, CH_2Cl_2); ^1H NMR (500 MHz, CDCl_3) δ = 5.15 (1H, dd, J = 5.8, 0.9 Hz), 4.70 (1H, d, J = 4.5 Hz), 4.35-4.28 (1H, m), 3.84 (3H, s), 3.72 (1H, s), 2.94 (s), 2.54 (1H, d, J = 11.3 Hz), 1.45 (3H, s), 1.33 (3H, s); ^{13}C NMR (126 MHz, CDCl_3) δ = 170.8 (s), 112.8 (s), 93.8 (d, J = 280.3 Hz), 85.4 (s), 72.5 (d, J = 3.1 Hz), 70.4 (d, J = 6.7 Hz), 64.3 (d, J = 23.5 Hz), 59.6 (d, J = 17.6 Hz), 53.4 (s), 28.1 (s), 26.8 (s); ^{19}F NMR (500 MHz, CDCl_3) δ = -146.5 (t, J = 5.3 Hz); ν_{max} (film) 3504, 3490, 2994, 2959, 1741, 1727, 1235, 1208, 1094, 1033, 858 cm^{-1} ; HRMS (ESI+) m/z calculated $(\text{C}_{11}\text{H}_{15}\text{O}_7\text{FNa})^+$ 301.0694, found 301.0709.

RESULTS AND DISCUSSION

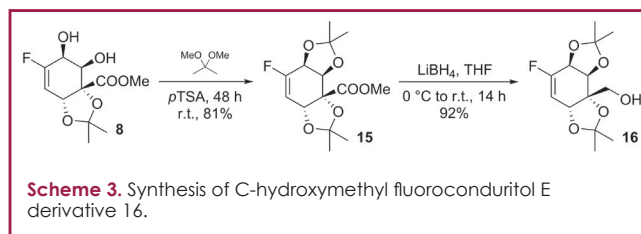
Several protocols have been reported previously (29) for the fermentative dearomatisation of 3 ($R = \text{H}$) to 4 ($R = \text{H}$). After consumption of starting material and removal of cellular material by centrifugation, the supernatant liquid is concentrated under reduced pressure and acidified to pH 3, so that the desired product (initially present in its ionised carboxylate form) is protonated to give carboxylic acid 4. This is then extracted into an organic phase (ethyl acetate). Care must be taken during the acidification step, since acid-mediated rearomatisation of the product occurs at low pH. Specifically, all arene *cis*-diols (2 and 4) can rearomatise through loss of water (which in the case of 4 would give salicylic acid), but an additional decomposition pathway is available to *cis* diols of type 4, namely dehydration with concomitant decarboxylation (to give phenol). In the case of *p*-fluorobenzoic acid 5, we initially adopted the protocol described above. Although complete conversion to desired product 6 was observed during fermentation (by direct ^{19}F -NMR analysis of the fermentation broth), after acidification and extraction, no 6 was isolated. Instead, *p*-fluorophenol was the sole product, implying complete decomposition of 6 by dehydration/decarboxylation during the attempted extraction. From this we infer that 6 is appreciably more acid-sensitive than 4 ($R = \text{H}$). Thus, we adopted an alternative reported protocol (31), whereby after concentration of the supernatant, isopropanol is used to precipitate the product directly as a mixed sodium/potassium salt. This crude product also contains various salts from the growth medium, but this circumvents the rearomatisation problem, and the product as a mixed salt may be stored in a freezer for prolonged periods. We assume that 6 is a single enantiomer of the absolute configuration shown, by analogy with 4 ($R = \text{H}$), whose enantiopurity and absolute configuration have previously been determined (29).

With 6 in hand as the mixed salt, we wished to explore selective functionalisations of the diene motif. To this end, the oxygen-containing functionality was protected, as shown in scheme 2a. Treatment of **6-Na/K** with 2,2-dimethoxypropane and TFA protected the diol as an acetonide, after which methyl ester formation with trimethylsilyldiazomethane gave fully protected 7. The first diene functionalisation we investigated was dihydroxylation catalysed by osmium tetroxide. For the previously known non-fluorinated analogue of 7, it is reported (29) that dihydroxylation favours the alkene distal to the ester in a ratio of 5:1, presumably due to steric repulsion from the ester substituent. However, due to the presence of the fluorine in 7, we observed the selectivity to be reversed in this case – dihydroxylation of the alkene proximal to the ester predominated, with 8 being formed as the major product (Scheme 2a). (In all cases dihydroxylation was observed on the β -face only, due to steric repulsion from the α -face acetonide).

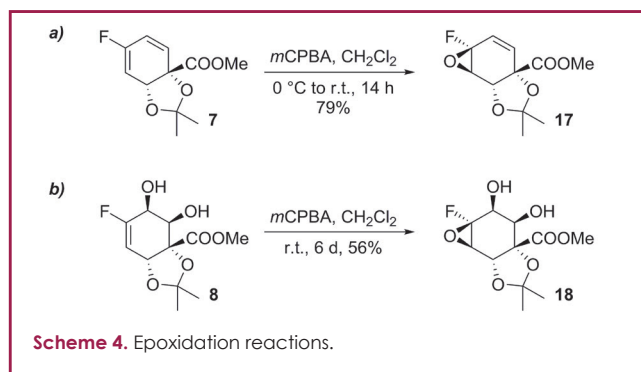


Clearly in the case of 7 the electronic deactivation of the fluoroalkene is the dominant effect determining regioselectivity. Nevertheless, small amounts of a byproduct were also isolated, shown by ^{19}F -NMR not to contain fluorine. The structure was assigned as cyclohexadienone 9 and a mechanistic proposal for its formation is shown in Scheme 2b. Thus, dihydroxylation of the fluoroalkene in 7 leads to α -fluoroalcohol 10, which spontaneously loses fluoride to give α -hydroxyketone 11. A second net oxidative transformation is required to produce 9 from 11, and we propose that 11 tautomerises to its enol form 12, which can be considered an extremely electron-rich olefin. This in turn undergoes dihydroxylation to give bis(ketone hydrate) 13. Loss of water then gives α -diketone 14, which tautomerises to 9. In support of this mechanistic proposal, OsO_4 -catalysed dihydroxylation of fluoroalkenes is a rare but precedented process (32), and dihydroxylation of 12 has at least circumstantial precedent (33).

Partially protected tetraol 8 is an ideal intermediate for elaboration to fluorocarbasugar targets. To this end, 8 was fully protected as bis(acetonide) 15, followed by ester reduction to give C-hydroxymethyl fluoroconduritol E derivative 16 (34). Both reactions proceeded in good yield (Scheme 3), and further transformations of 16 will be reported in due course.



The second diene functionalisation we investigated was epoxidation. Treatment of protected diene 7 with *m*CPBA gave exclusive epoxidation of the fluoroalkene on the less hindered β -face to give 17 (Scheme 4a). The selectivity of this process is once again different from that for the non-fluorinated analogue of 7, where epoxidation of both alkenes reportedly occurs (29). The unique steric and electronic properties of fluorine atoms in stereoselective epoxidation reactions of fluoroalkenes have been reported elsewhere (35). The resultant α -fluoroepoxides, as found in 17, are a known functional group whose reactivity towards nucleophiles has been documented (36), but which remain underexploited in synthesis to date. It should also be noted that no spontaneous rearrangement of the epoxide in 17 was observed. This is in contrast to the case of the *ortho,meta*-diol 2 ($\text{R} = \text{F}$), whose epoxidation furnishes the corresponding allylic fluoroepoxide, which in turn spontaneously rearranges to a dihydrofuran (37). In comparison with 7, epoxidation of partially protected tetraol 8 was much slower (Scheme 4b), furnishing epoxide 18 in only 56% yield (unoptimised) after 6 days. (Synthesis of 18 by dihydroxylation of 17 was also attempted but proved to be even more sluggish). Compound 18 possesses 6 contiguous stereocentres, including two quaternary centres, and is derived from achiral fluoroaromatic starting material 5 in just five steps. We contend that the first (biocatalytic) step is the most significant, since stereochemical information imparted by the enzyme allows for subsequent stereocentres to be introduced under substrate control in later steps. Thus, all stereochemistry in 18 is ultimately of enzymatic origin.



CONCLUSIONS

In preliminary experiments we have demonstrated the synthetic versatility of arene *cis*-diol building block 6 derived from *p*-fluorobenzoic acid (5) by arene dioxygenase-mediated oxidation. Despite being near isosteric with hydrogen, the fluorine substituent exerts a significant electronic effect on the path of subsequent transformations of 6, allowing access to unusual structural motifs in short order. It is especially noteworthy that two oxidative processes (dihydroxylation and epoxidation), which both employ electrophilic oxidants, occur preferentially at *different alkenes* in the same fluorodiene. Whereas the

deactivating electronic effect of the fluorine renders the non-fluorinated alkene more electron rich, it may be that the ability of fluorine to hydrogen bond with the peracid reagent leads to a directed epoxidation of the less electron rich alkene, thus explaining the regiodivergence of dihydroxylation versus epoxidation. Further investigations into the chemistry of **6** are ongoing and will be reported in due course.

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